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# Factor H Cleavage by the *Treponema denticola* Protease Dentilisin: Understanding the Pathogenesis of Periodontal Disease

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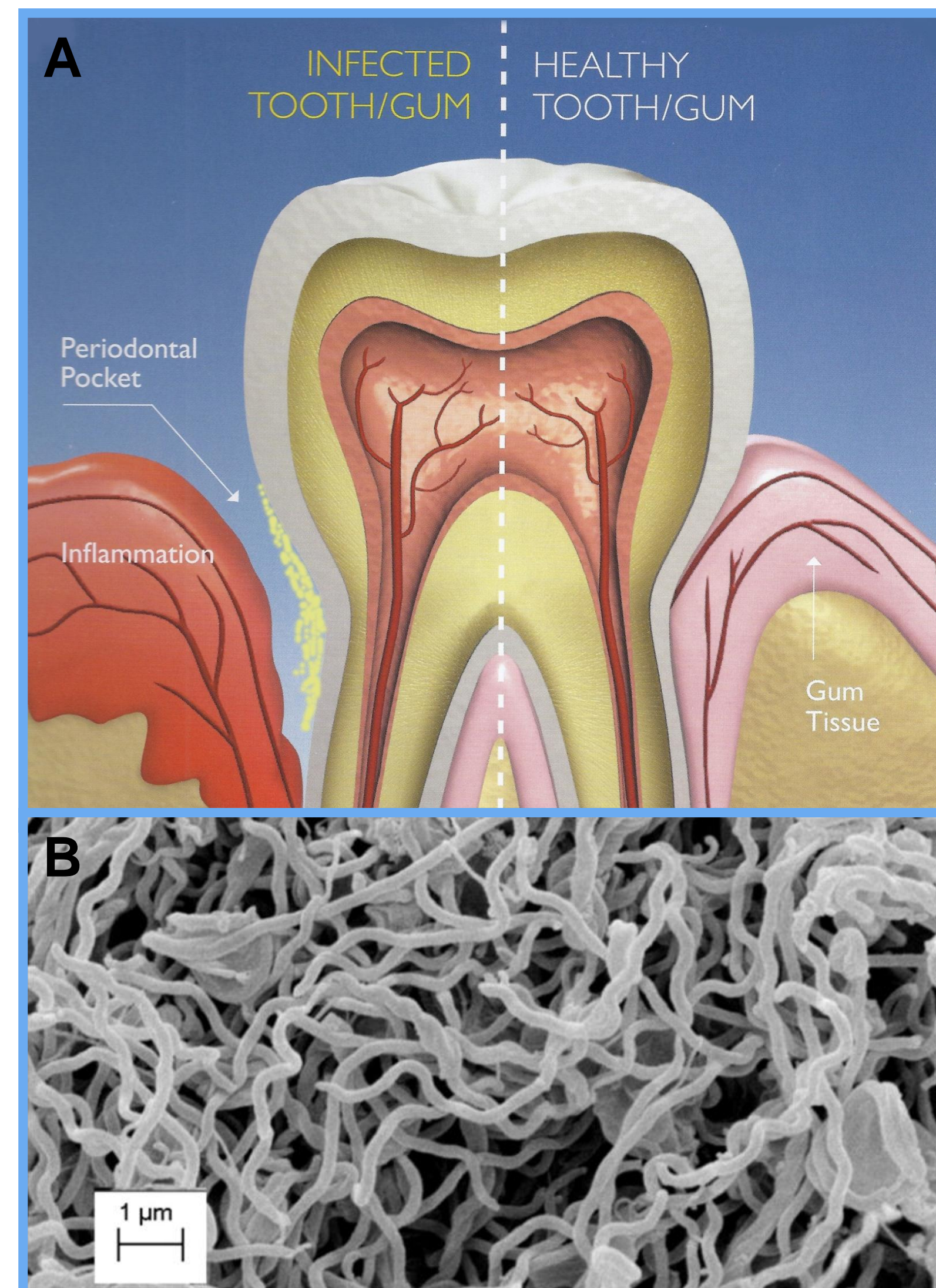
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## Introduction

Periodontal disease is an infectious condition that results in the inflammation and gradual degradation of gum tissue and alveolar bone. Caused by plaque deposits on teeth that harden into tartar to inflame gum tissue, the condition is highly pervasive, with 15 percent of US adults over 30 years of age exhibiting destructive periodontal disease. The abundance of *Treponema denticola*, a spirochete, in the oral flora is strongly correlated with disease severity. *T. denticola* binds to Factor H (FH), a negative regulator of the complement system, through its Factor H binding protein (FhbB) to evade complement-mediated killing. The protease dentilisin, produced by *T. denticola*, cleaves FH for reasons that are still being studied. We hypothesize that dentilisin-dependent cleavage of FH may result in the local dysregulation of the complement immune system, leading to gum destruction. Characterization of the complex interaction between *T. denticola* and FH may explain the molecular pathogenesis of periodontal disease.



**Figure 1:** A) Periodontal disease is a chronic inflammatory disease that is the leading cause of tooth loss worldwide. B) *Treponema denticola*, a spirochete, is highly correlated with periodontal disease severity.

## Goals

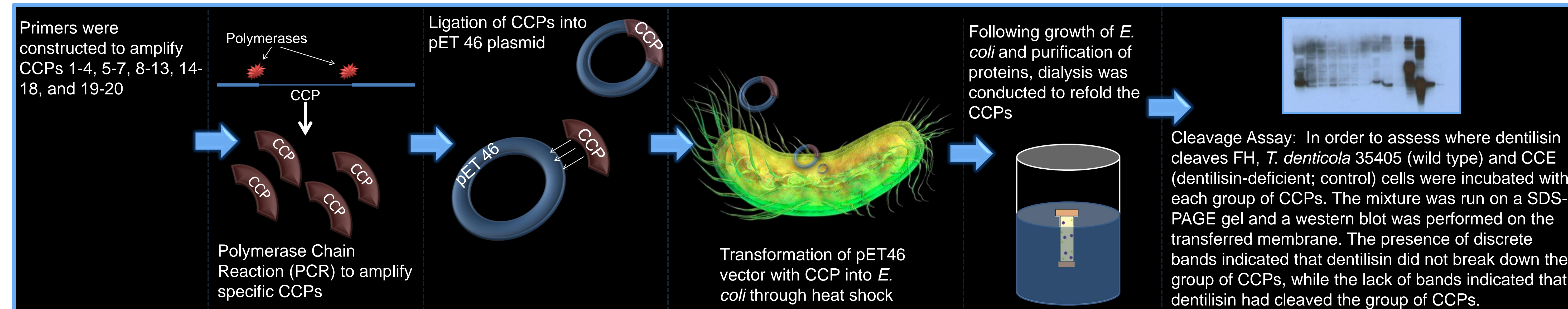
**Primary Goal: To identify the specific location of FH cleavage by dentilisin.**

- FH is divided into twenty Complement Control Protein domains (CCPs). We will determine within which group of CCPs dentilisin cleaves FH.

- We will accomplish this goal by assessing the following:

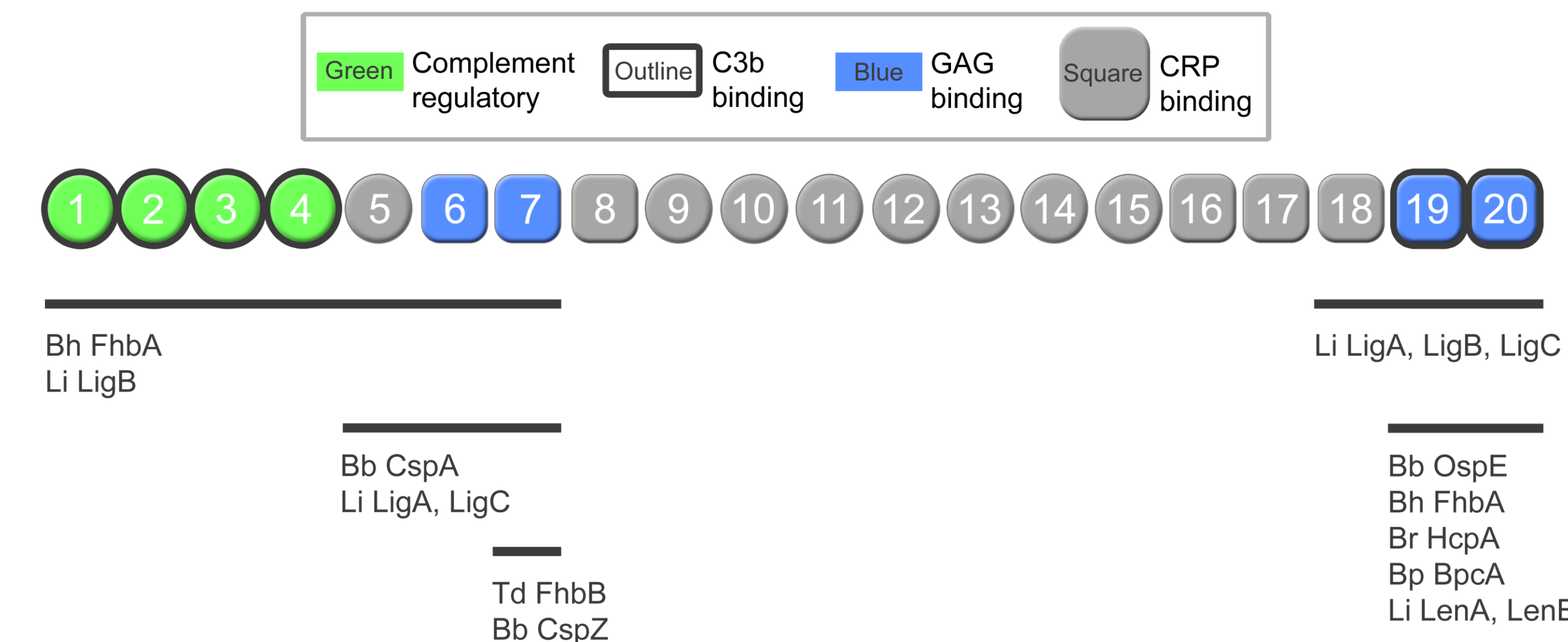
1. CCP Functionality
2. CCP Binding
3. Cleavage of groups of CCPs by dentilisin after each group of CCPs is incubated with *T. denticola* cells

## Methods

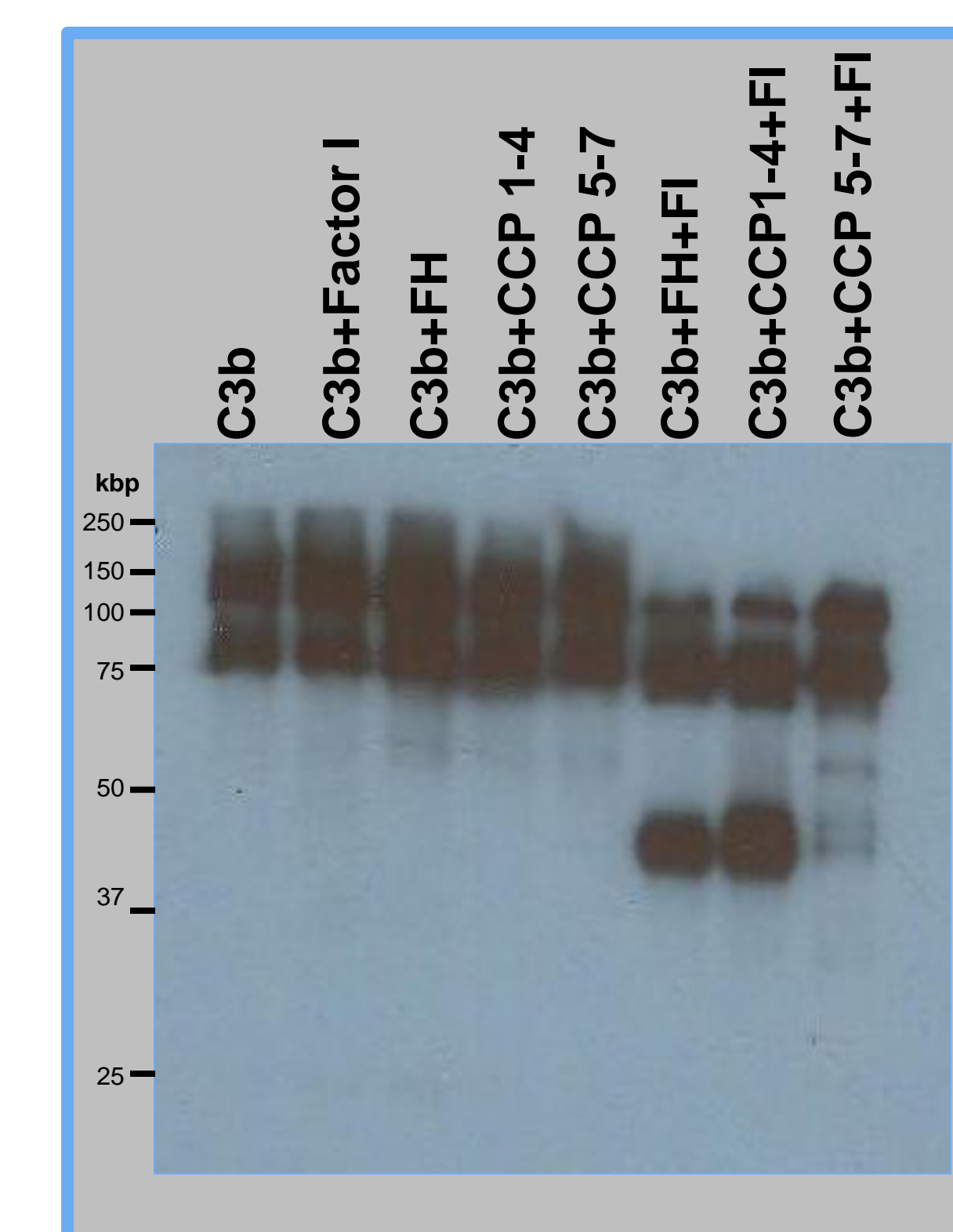


**Figure 2:** Procedures used for determining the location of FH cleavage by the protease dentilisin

## Results: Assessment of CCP Functionality

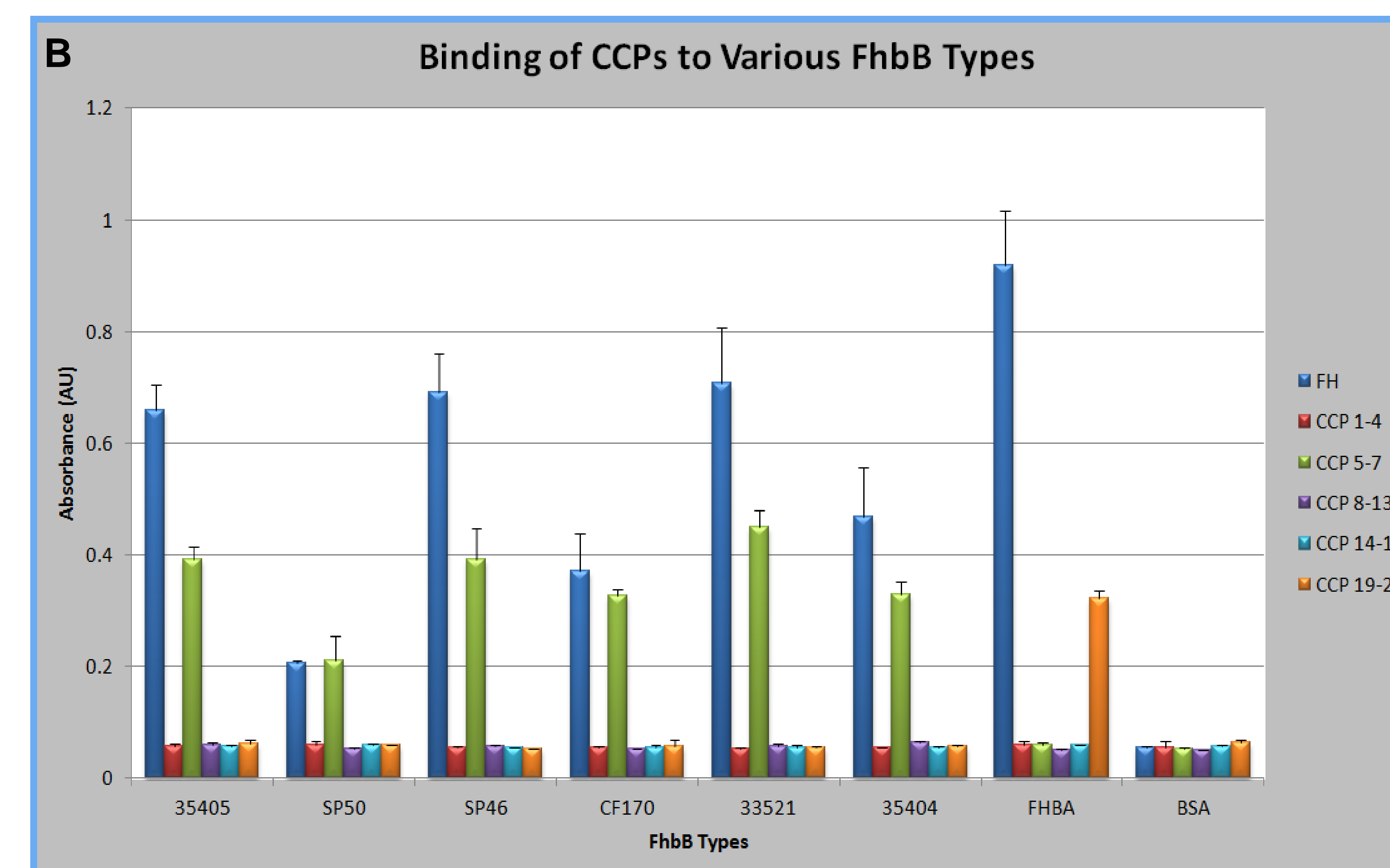
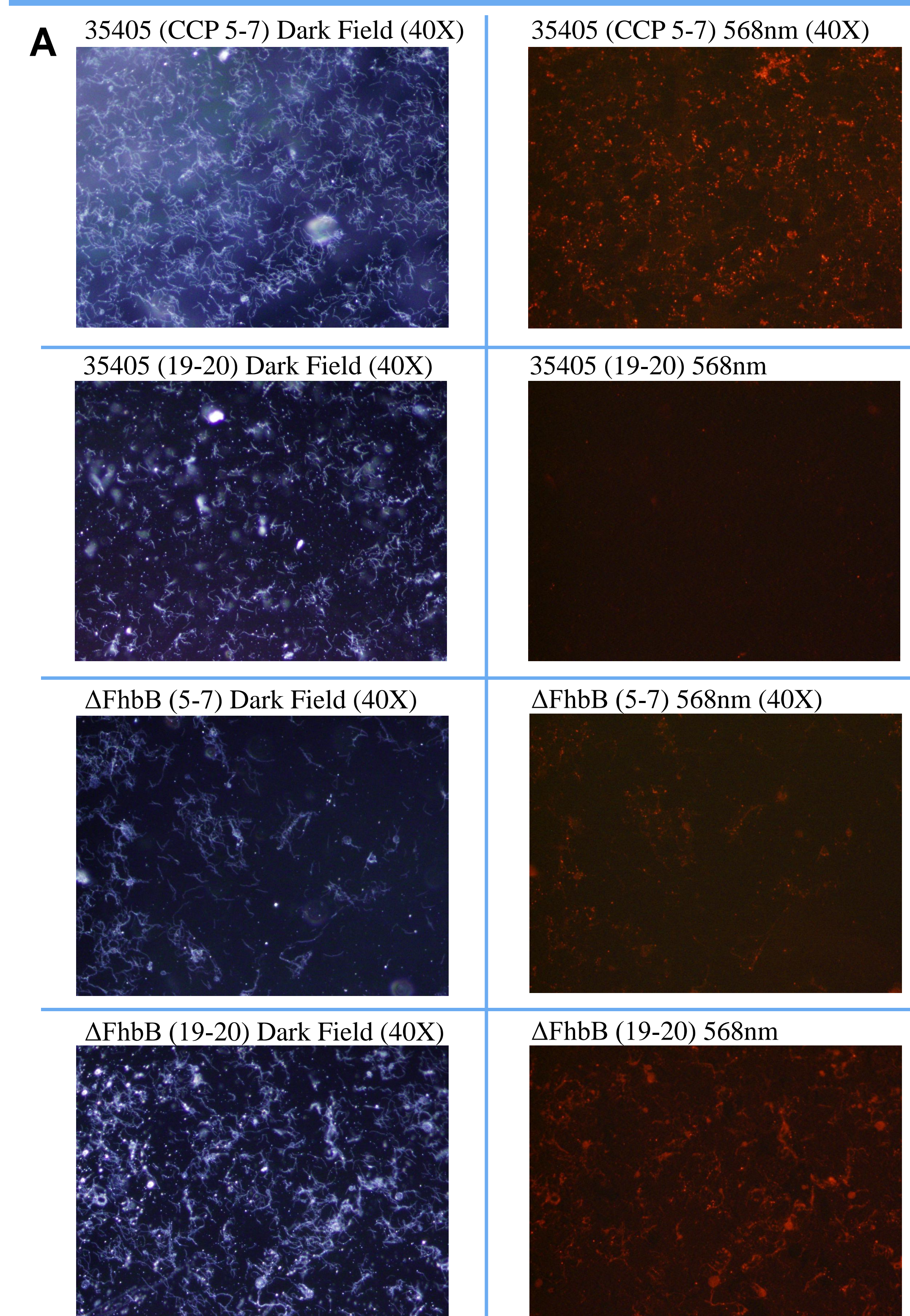


**Figure 3:** Diagram depicting CCP domains of FH and their varying activities. CCP 1-4 contains complement regulatory activity and FhbB binds to CCP 6-7. Spirochetal FH binding proteins interact as shown.



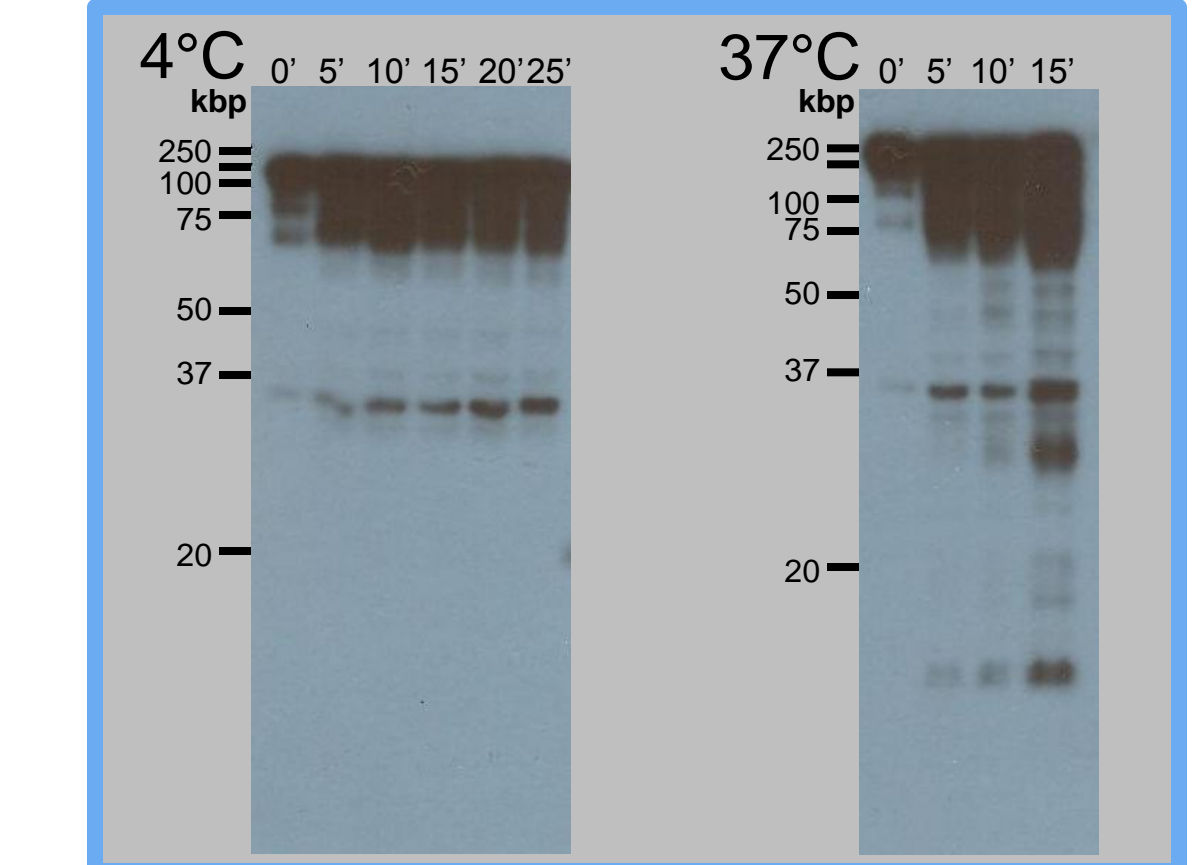
**Figure 4:** Assessment of CCP 1-4 cofactor activity. A western blot was conducted to assess the activity of CCP 1-4 and confirm proper refolding during dialysis. CCP 1-4 has previously been known to act as a cofactor in C3b cleavage when derived from a eukaryotic host. Because we used a prokaryotic organism, *E. coli*, to generate our CCP constructs, we confirmed that activity of CCPs was not lost. As the western blot shows, CCP 1-4 did indeed cleave C3b when compared to cleavage of C3b by full length FH. Thus, CCP refolding was successful and activity was not lost. CCP 5-7, a control, did not cleave C3b, as was expected.

## Results: Assessment of CCP Binding

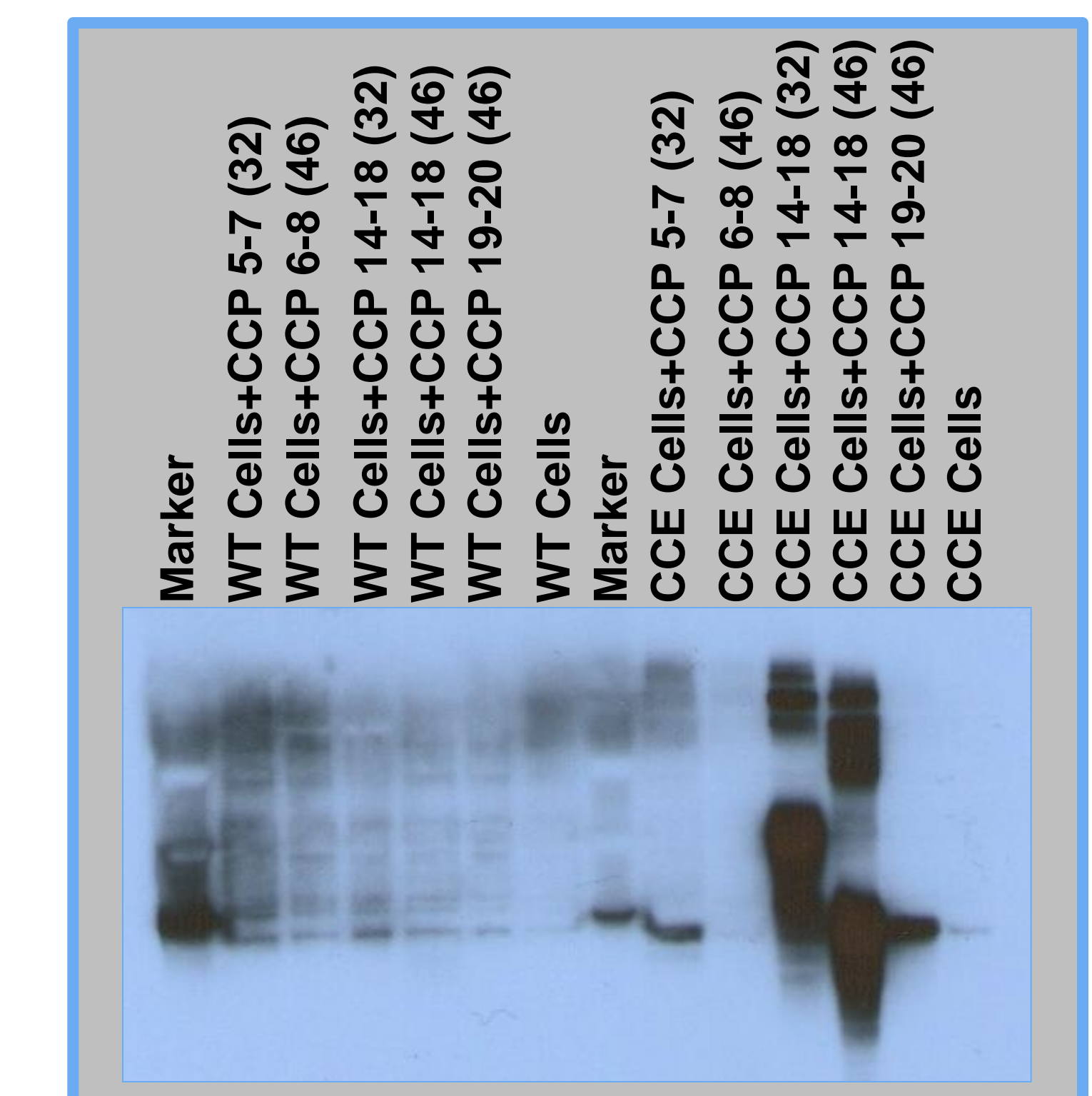


**Figure 5:** A) CCP binding to whole *T. denticola* cells. Using immunofluorescence assays, we were able to visualize binding of CCPs 5-7 and 19-20 to two *T. denticola* strains. In the 35405 (wild type) strain, binding of CCP 5-7 occurred but not of CCP 19-20. In the ΔFhbB strain (FhbB-deficient), no significant binding of both CCPs 5-7 and 19-20 occurred. However, there seems to be some non-specific binding of the ΔFhbB strain to CCP 19-20 that may require different experimental techniques. B) CCP binding to FhbB types. An ELISA was conducted in order to confirm binding of CCPs to FhbB proteins from various *T. denticola* strains. Based on previous lab results, it was expected that CCP 5-7 and FH will bind to all the FhbB types and CCP19-20 will bind to fhbA, another FH binding protein, as the results show. Bovine serum albumin (BSA) was used as a control.

## Results: Cleavage Assay



**Figure 6:** Time-course western blots. In preparation for the cleavage assay, FH was incubated at two different temperatures (4°C and 37°C) and several different times to determine the conditions necessary for dentilisin to completely cleave FH. At 37°C, dentilisin has cleaved FH after 5 minutes, whereas at 4°C, dentilisin has still not completely cleaved FH even after 25 minutes. To ensure appropriate conditions are allowed for dentilisin to cleave the CCP constructs, we chose to conduct our cleavage assay at 37°C for 30 minutes (shown below).



**Figure 7:** Results of the cleavage assay. CCP constructs 6-8, 14-18, and 19-20 from the pET 46 vector were incubated with either strain 35405 (wild type) or CCE (dentilisin-deficient) for 30 minutes at 37°C. From this, it can be seen that after incubation with the 35405 strain, dentilisin has cleaved all of the CCP constructs. In the CCE strain, the CCP constructs are intact because dentilisin was not present to cause cleavage. CCP constructs 5-7 and 14-18, which were derived from a pET 32 vector, were also used in this cleavage assay and produced similar results.

## Summary

- This is the first time that functional FH fragments were successfully cloned, expressed, and refolded in *E. coli*
- Similar to full length FH, all of the CCP constructs tested were degraded in a dentilisin-dependent mechanism
- The CCPs were completely degraded with no detectable fragments, indicating the presence of multiple cleavage sites that may explain the rapid kinetics of proteolysis
- Cleavage of FH by dentilisin may decrease FH levels locally, causing complement dysregulation and thus the gum and bone destruction that characterizes periodontal disease. By understanding the action of dentilisin in FH cleavage, we now have a better picture of the molecular pathogenesis of periodontal disease.